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Enzymatic Method Measurement of Serum Amylase and Lipase

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An abbreviated version of this protocol was published in Frontiers in Immunology in May 2019

TLR3 Ligand PolyI:C Prevents Acute Pancreatitis Through the Interferon-β/Interferon-α/β Receptor Signaling Pathway in a Caerulein-Induced Pancreatitis Mouse Model

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Detailed protocol

Enzymatic Method Measurement of Serum Amylase and Lipase

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1.Sample preparation

Whole blood from control and experimental acute pancreatitis mice were centrifuged at 1,500 g for 10 min at 4℃ to separate the serum. If you cannot perform the assay immediately, the serum samples should be aliquoted and stored at -80°C.

2.Measurement of serum amylase activity

(1) We recommend performing several dilutions of the samples to ensure the OD_{660} values are within the linear range (mild acute pancreatitis: 1:50-1:100 dilution; severe acute pancreatitis: 1:500-1:1000 dilution).

(2) Assay procedure:

	Sample tubes (mL)	Blank tubes (mL)
Amylase Substrate Mix (Preheat at 37℃ for 5 min)	0.5	0.5
Serum samples	0.1	
Mix thoroughly and incubate the plate at 37 °C for 7.5 min		
Amylase Assay Buffer	0.5	0.5
ddH_2O	3.0	3.1
Mix thoroughly, and measure absorbance at OD_{660} in a 96-well clear plate.		

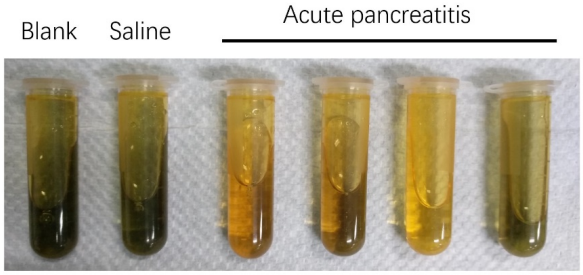
(3) Activity of amylase is calculated as:

Amylase activity (U/dL) = $(OD_{Blank}-OD_{Sample})/ OD_{Blank}$ * 80 * sample dilution fold

(4) Solution:

Amylase Substrate Mix	0.4 mg/mL amylon solution
Amylase Assay Buffer	0.01 mol/mLiodine solution (keep away from light)

(5) Results example:



3. Measurement of serum lipase activity

(1) We recommend performing several dilutions of the sample to ensure the OD_{580} values are within the linear range (mild acute pancreatitis: no dilution; severe acute pancreatitis: 1:5-1:10 dilution).

(2) Assay procedure:

	Blank wells	Standard wells	Sample wells
ddH_2O (μL)	4		
Standard Sample (μL)		4	
Serum samples (μL)			4
Reagent 1 (μL)	200	200	200
Mix thoroughly and incubate the plate at 37 °C for 3~5 min			
Reagent 2 (μL)	50	50	50
Mix thoroughly, incubate the plate at 37 °C for 2 min, and measure absorbance immediately at OD_{580} in a kinetic mode, every 2 minutes. $\Delta A_{580nm} = A2 - A1$			

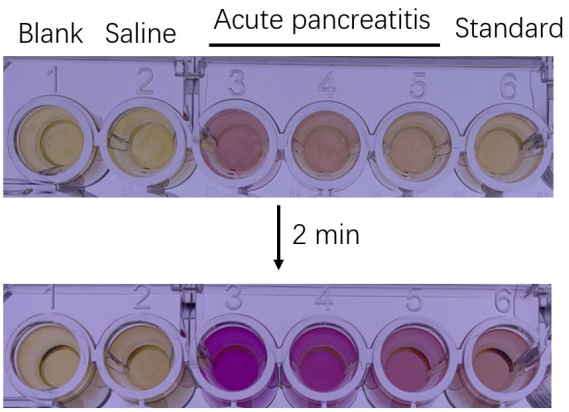
(3) Activity of lipase is calculated as:

Lipase activity (U/L) = $(\Delta A_{Sample}-\Delta A_{Blank})/ (\Delta A_{Standard}-\Delta A_{Blank})$ * The activity of Standard sample * sample dilution fold

(4) Solution:

Reagent 1	Containing Sodium taurocholate and calcium chloride
Reagent 2 (Lipase Substrate)	1,2-Di-O-lauryl-*rac*-glycero-3-(glutaric acid 6-methylresorufin ester)
Standard Sample	45.8 U/L

(5) Results example:



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How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Chen, S. and Ma, F. (2021). Enzymatic Method Measurement of Serum Amylase and Lipase. Bio-protocol Preprint. bio-protocol.org/prep1240.
2. Huang, C., Chen, S., Zhang, T., Li, D., Huang, Z., Huang, J., Qin, Y., Chen, B., Cheng, G., Ma, F. and Zhou, M.(2019). TLR3 Ligand PolyI:C Prevents Acute Pancreatitis Through the Interferon-β/Interferon-α/β Receptor Signaling Pathway in a Caerulein-Induced Pancreatitis Mouse Model. Frontiers in Immunology 10. DOI: 10.3389/fimmu.2019.00980